Comm.
Dr. Bing
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Dr. Sommers
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PULMONARY

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-6885

Application for Research Grant
(Use extra pages as needed)

Date: July 31, 1973

1. Principal Investigator (give title and degrees):
Branislav Vidic, S.D., Assoc. Prof. Anatomy; Director of Project
Paul Hamosh, M.D., Ass't. Prof. Physiology & Biophysics and Medicine; Co-Principal Investigator
Henry Yeager, M.D., Ass't. Prof. Medicine; Consultant
Margit Hamosh, Ph.D., Consultant

2. Institution & address:
Georgetown University School of Medicine
3900 Reservoir Road, N.W.
Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:

4. Short title of study:
The Effect of Cigarette Smoke on Lung Metabolism

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

The aim of this research is to study the effect of cigarette smoke, filtered and unfiltered, on the biosynthesis and function of surfactant and connective tissue (elastin and collagen). An integrated approach, using the tools of physiology, morphology and biochemistry is presented. Rats will be chronically exposed to cigarette smoke and then subjected to three main experimental designs. The first aims to study the incorporation and turnover of labeled substrates presented to the intact animal. The second design calls for isolation of the lung into a ventilated, perfused preparation. This preparation can be "stressed" both mechanically (forced ventilation) and biochemically (substrate deprivation). We shall study the effect of these acute "stresses" on the biosynthesis and turnover of substrates in lungs of chronically smoked and control rats. The third design involves isolation of the great alveolar cell and the study of in vitro surfactant synthesis.

The major objective of this research is to establish the site, sequence and extent of tobacco smoke effect of the biosynthetic pathways responsible for the maintenance of integrity of structure and proper function (elastic recoil) of the lungs.

8. Brief statement of working hypothesis:

Rats exposed to chronic cigarette smoke might show detectable changes in 1) biosynthesis of surfactant; 2) biosynthesis of elastin and collagen; 3) chemical and functional response patterns to acute mechanical or chemical stress.

9. Details of experimental design and procedures (append extra pages as necessary)

See appended.

9. Background, details of experimental design and procedure

GENERAL BACKGROUND

The effect of cigarette smoke on human and animal lung has been widely investigated. It has been shown that chronic exposure to cigarette smoke will result in changes in the airways: chronic bronchitis (1). The link between smoking and emphysema is less well documented. The frequent association between chronic bronchitis and emphysema in smokers (2) suggests some causal relationship. The present tendency is to shift the initial changes occuring in the pathogenesis of chronic obstructive lung disease further toward the periphery of the lung, mainly the small airways (3). Little work has been done on the effect of cigarette smoke on the function of the alveolar epithelium or the biosynthesis of surfactant, elastin and collagen. Quoting from the Task Force Report on Respiratory Diseases of the National Institutes of Health (4):

"Information is badly needed concerning the mechanism of cigarette smoking damage. Here, it would be well to take advantage of the many advances in cellular biology that have been made in the past ten years. Some such studies are already in progress, including investigations concerning clearing mechanisms, ciliary action , and quantitative studies of bronchial cellular change. What have not been looked at carefully have been the determinants of individual response, including metabolism of cigarette smoke products, enzyme effects, variations in tissue repair."

In their conclusion it is also stated: "Research should be addressed to fundamental studies of the mechanism of damage from cigarette smoking..."

SPECIFIC BACKGROUND

The great alveolar cell (type II) in the alveoli is a secretory cell, whose main function is the biosynthesis and secretion of surfactant, a substance essential for the proper function of the lung as a mechanical pump. The elastic behavior of the lung is dependent on two factors: 1) adequate or functionally effective surfactant and 2) integrity of the connective tissue components in the alveolar wall, such as collagen and elastin. Very little is known on the effect of smoke on surfactant function and synthesis and practically nothing of its effect on the biosynthesis of connective tissue.

1. Effect of cigarette smoke on surfactant function and synthesis

   a. Cigarette smoke alters the surface characteristics of lung extract (5,6,7,8)

   b. The biosynthesis of lecithin was studied in cigarette smoking dogs (9). This study is unacceptable as a good

physiologic study because of the large dose and short duration of cigarette exposure. Nevertheless, it showed a significant decrease in choline and phosphate incorporation into lung lecithin.

c. The removal of used-up surfactant by alveolar macrophages and the effect of smoking on this process has attracted wide attention lately. However, this problem is outside the scope of our intended investigation.

2. The effect of cigarette smoke on connective tissue metabolism of the lung. This area of investigation is unexplored. Only the level of hydroxyproline, a marker of collagen metabolism (10) and a nonspecific "biochemical screen" (11) were measured after whole cigarette smoke exposure in mice. Unfortunately, the information about the normal biosynthesis of elastin and collagen in lung tissue is at best sketchy (12).

SIGNIFICANCE

Maximum expiratory flow, the major mechanical determinant of lung function is directly proportional to lung recoil pressure and inversely proportional to airway resistance. Lung recoil pressure is determined by surface tension which contributes about two thirds, and tissue elastic recoil which contributes about one third to it. Lung recoil pressure is also a function of lung inflation and the elastic properties of the lung are expressed in terms of volume/pressure relationship (lung compliance). Surface tension is modified by the presence of a surface active layer in the alveoli, called surfactant. This substance is responsible for the variation of surface tension with volume. Surface tension increases with lung volume and drops significantly at low lung volume. This mechanism is essential to prevent the collapse of lung regions (atelectasis) at the end of each expiration and therefore constitutes an essential mechanism for survival (13).

In emphysema, lung recoil is severely decreased by impaired tissue elasticity and probably by altered surfactant. The basic question is: What are the factors operative in causing these changes? In addition to this basic question we pose an additional one: Is cigarette smoke a primary, a contributory or an innocent factor in the pathogenesis of emphysema? These fundamental questions have not been answered by previous research. If cigarette smoke does have an effect, the mechanism is still unclear. This research is a step by step integrated approach to answer these questions.

References

1. The Health Consequences of Smoking. A Report of the Surgeon General, U.S. Department of Health, Education and Welfare. U.S. Government Printing Office, 1971.

2. N.L. Jones, B. Burrows, and C.M. Fletcher. Serial studies of 100 patients with chronic airways obstruction in London and Chicago. Thorax 22:327, 1967.

3. J.C. Hogg, P.T. Macklem, and W.M. Thurlbeck. Site and nature of airway obstruction in chronic obstructive lung disease. New Eng. J. Med. 278:1355, 1968.

4. Respiratory Diseases: Task Force Report on Problems, Research Approaches and Needs. The Lung Program, National Heart and Lung Institute, October 1972. DHEW Publication no. (NIH) 73-432.

5. W.A. Cook and W.R. Webb. Surfactant in chronic smokers. Ann. Thoracic Surg. 2:327, 1966.

6. S.T. Giammona. Effects of cigarette smoke and plant smoke on pulmonary surfactant. Am. Rev. Resp. Dis 96:539, 1967.

7. D. Miller and S. Bondurant. Effects of cigarette smoke on the surface characteristics of lung extracts. Am. Rev. Resp. Dis. 85:692, 1962.

8. S.A. Pratt, T.N. Finley, M.H. Smith and A.J. Ladman. A comparison of alveolar macrophages and pulmonary surfactant (?) obtained from the lungs of human smokers and nonsmokers by endobronchial lavage. Anat. Rec. 163:497, 1969.

9. J.A. Balint, S. Bondurant and E.C. Kyriakides. Lecithin biosynthesis in cigarette smoking dogs. Arch. Intern. Med. 127:740, 1971.

10. H. Rosenkrantz, H.J. Esber and R. Sprague. Lung hydroxyproline level in mice exposed to cigarette smoke. Life Sciences 8:571,1969.

11. H. Rosenkrantz and R. Sprague. Biochemical screen to investigate whole smoke and vapor phase effect in mice. Arch. Environ. Health 18:917, 1969.

12. R.G. Crystal, K.H. Bradley, and S.D. McConnell. Changes in lung collagen synthesis with age. Annual Meeting of American Thoracic Society, May 21-23, 1971 (abstract).

13. J. Mead. Mechanical properties of lungs. Physiol. Rev. 41:281, 1961.

TIMETABLE

General. All of the equipment except for the exposure apparatus and most methods in design one and two are already operational.

First Year. Recruitment of personnel, establishment of experimental routines and assembly of exposure apparatus will take between three and six months. However, work will proceed on acquiring basic information about lung metabolism (such as present in appendix I). The second half of the year will be used for studies of design one and preliminary studies of design two.

Second Year. Most of the work will be on design two (i.e. using the isolated/perfused lung preparation). The effect of forced ventilation, substrate deprivation, perfusion pressure etc. in smoked and control lungs will be investigated. Design three will be developed.

Third Year. Mostly work on isolated type II cells on the biosynthesis, location, storage and secretion of surfactant.

EXPERIMENTAL DESIGN

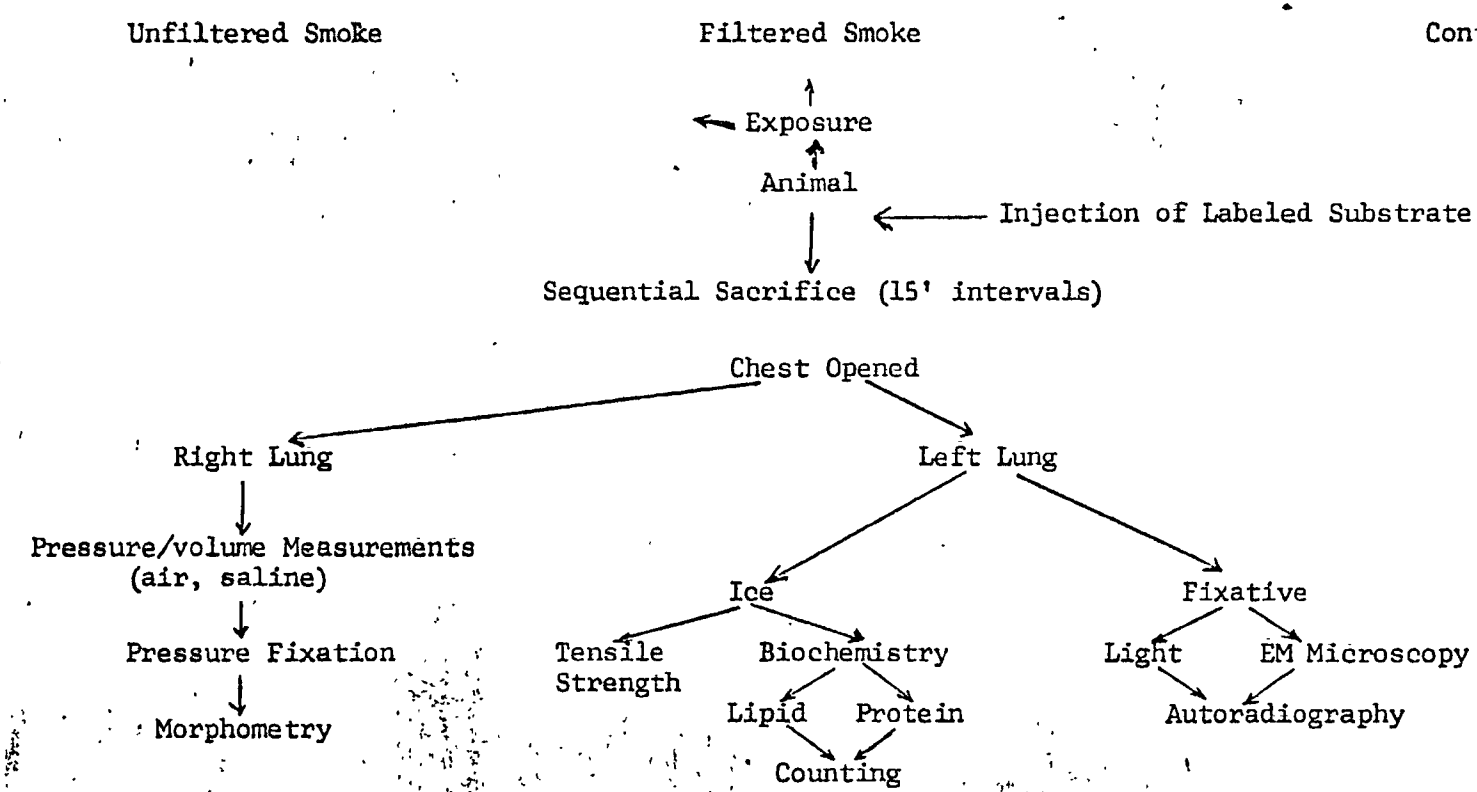
1. Selection of experimental animals. The material for this study will consist of caesarian-delivered adult male Sprague-Dawley rats. This species has been selected because of convenience for purchasing and maintenance. Caesarian delivered rats have a far lower incidence of lung infection than normally delivered. The animals will be individually maintained for at least two weeks in an isolated chamber under optimal conditions (with controlled humidity and temperature) and on laboratory Purina Chow diet ad libitum. Healthy animals without signs of respiratory disease will be divided into three groups: experimental, sham control and cage control. The experimental group will be further subdivided into a number of subgroups. Each subgroup consisting of six animals will be exposed to cigarette smoke for differently designed periods of time. Two other subgroups of six animals, sham and cage controls respectively, will be considered in relation to every experimental subgroup.

2. Exposure to cigarette smoke. In order to make anticipated results of this proposal better comparable to other similar studies, we are proposing that the "smoking machine" be supplied from the Council for Tobacco Research - U.S.A. In case that such an arrangement cannot be made, we will construct our own device that would best fulfill Requirements For Any Mechanical Arrangement For Exposure Of Animals To The Inhalation Of Cigarette Smoke Under Conditions Comparable To Those of Human Smoke Exposure. The "reference" cigarette from the University of Kentucky will be used

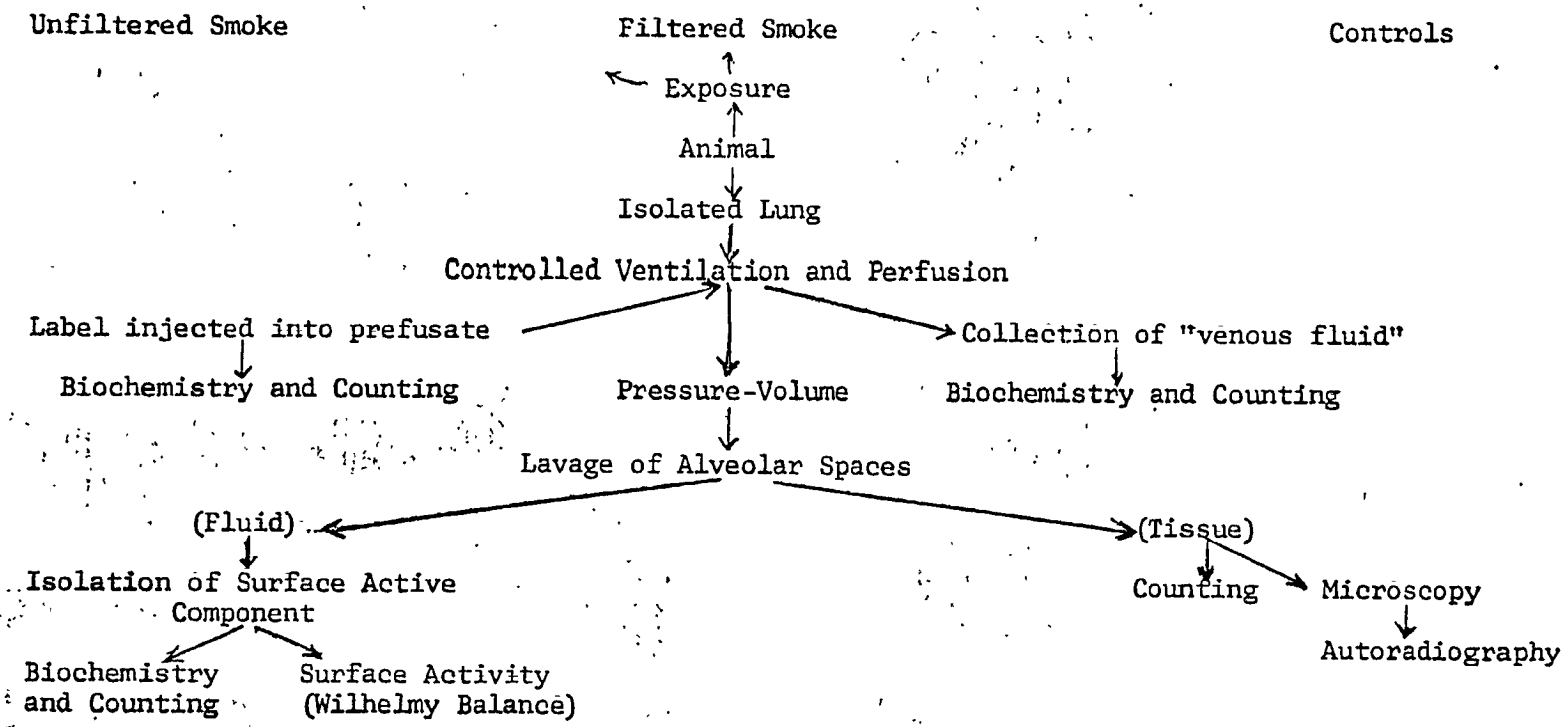
for the entire project. Some experimental subgroups will be exposed once daily (9 a.m.) for one hour, others twice daily (9 a.m. and 5 p.m.) for one hour to whole cigarette smoke. Still other subgroups will be exposed to cigarette smoke filtered through a Cambridge filter. Sham control subgroups will be enclosed in the same chamber through which the room air instead of cigarette smoke will be circulated for the same lengths of time as for the corresponding experimental subgroups. Since the mode of exposure has not been finalized the duration of exposure will be determined after resolution of this question.

3. Experimental design, schematic presentation. We propose three approaches for this study. The first approach (design one) is designed to study the dynamics and morphology in the intact animal. The second approach (design two) utilizes our ability to keep the lungs viable and functioning in an isolated system. This system provides controlled ventilation and perfusion. It also provides the opportunity to "stress" these lungs by forced ventilation, by substrate deprivation etc. This enables us to compare the performance of lungs from smoked and control rats under adverse conditions and thus test "in vitro" multiple etiology. The third approach (design three) involves the separation and isolation of pure type II cells. Whereas the previous two techniques are available at least partially to us, the third approach has not been developed yet and needs to be introduced. It certainly promises to be the method of choice in analyzing processes at the cellular level.

EXPERIMENTAL DESIGN ONE



EXPERIMENTAL DESIGN TWO

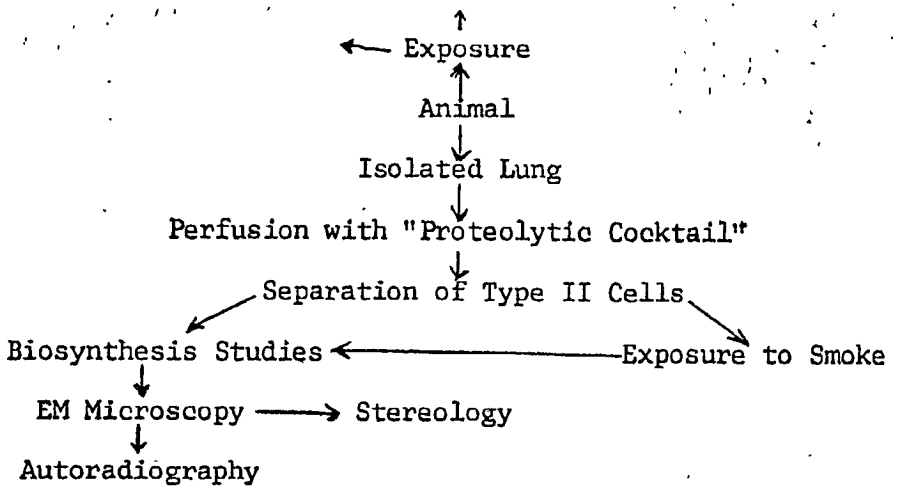


EXPERIMENTAL DESIGN THREE

Unfiltered Smoke

Filtered Smoke

Controls



It is not implied that all steps will be incorporated in every sequence of experiments. Each design could be performed in several stages. This will depend on logistics and skill of the investigators and their assistants. However, this team of investigators have already performed work of this nature (Hamosh and Hamosh. Lung lipoprotein lipase, effect of starvation. See appended manuscript.)

NARRATIVE DESCRIPTION OF EXPERIMENTAL DESIGNS

A. Design One

Animals (controls and exposed) will be injected into the tail vein with radioactive labeled substrates, mostly C^{14} and H^3 palmitate and leucine (see procedure). The chest will be opened and the specimen obtained from the left lung and distributed in appropriate fixatives for EM or frozen (cooled) and processed for extraction, homogenization or sliced. The right main bronchus will be then cannulated and the lung inflated to the desired transpulmonic pressure and the deflation pressure volume relationship measured. Following this the lung will be pressure fixed with formaldehyde, embedded and sliced. The microscopic slides will be subjected to morphometric and autoradiographic evaluation.

B. Design Two

The animal will be anesthetized by intraperitoneal injection of Pentobarbital and the trachea cannulated. The pulmonary artery will be cannulated through the right ventricle and the left atrium cannulated for collection. The specimen will be transferred into the box designed for controlled perfusion and ventilation at 37°C. It is also equipped for continuous monitoring of transpulmonic pressure, so that the pressure/volume relationship can be monitored. After establishing a baseline under controlled conditions, the labeled substrate is added to the perfusate. The "venous" part of this non-circulating perfusion will be collected in test tubes at equal time intervals and prepared for the scintillation counter. Comparing the activity of the perfusate to the activity of the sequential collection, the dynamics of the turnover of the labeled substance can be determined by calculation. Surfactant will be both present in the tissue and secreted to the alveolar surface. Therefore, at the termination of perfusion the lung will be "washed out" by saline to harvest the surface active fraction and determine the radioactivity. Specimens of the tissue will be taken for autoradiography and extraction of lipid, to determine the activity and its location in the tissue.

C. Design Three

This design calls for the separation and harvest of individual type II cells and their isolation in relatively pure and viable short term cultures. The feasibility of this has been demonstrated by Gould et al.* To manipulate pure cell population is ideal for determining the dynamics of intracellular processes. It is possible to study the comparative behavior of cells from smoked vs. non-smoked animals. It would also be of great interest to expose these cells in vitro to cigarette smoke.

PROCEDURES

A. Electron Microscopy

Ultrastructural Methods. Tissue samples from lungs in vivo (design one), perfused lungs (design two) and in vitro cultured cells (design three) will be fixed in an aldehyde mixture (1) for two hours at 4°C and washed overnight in an appropriate buffer. Following the post-fixation in one percent osmium tetroxide, the samples will be dehydrated, embedded in Epon and sectioned with a diamond knife in 300-600 A° thin sections. Some grids (designs one, two and three) will be treated conventionally with heavy metals, whereas the other grids (designs one and two) will be processed according to phosphotungstic acid (10% aqueous solution, pH 1.5) procedure of Kay (2) and silver tetraphenylporphinesulfonate technique of Albert and Fleischer (3) for demonstration for collagen and elastin fibers respectively.

Autoradiographic Methods (4). Tissue samples from lungs in vivo (design one), perfused lungs (design two) and in vitro cultured cells (design three) will be fixed in a 2% osmium tetroxide buffer with veronal acetate to pH 7.2 for two hours at 4°C and washed overnight in an appropriate buffer. Following the block staining in 1% uranyl acetate, dehydration and embedding in Epon, tissue samples will be sectioned with a diamond knife in 600-900 A° thin sections and mounted on grids previously coated with collodion film baked by a thin carbon layer. The grids will be exposed to the photosensitive emulsion (Ilford L-4) diluted one to four with distilled water for approximately six months, developed in Microdal X for 5 minutes, washed, fixed in Kodak rapid fixer for 5 minutes and washed again in running and distilled water for 5 and 2 minutes respectively.

Electron microscopy and photography will be carried out with the aid of an AEI-EM 8 electron microscope.

* Science 178:1209, 1972

References

1. S. Ito and M.J. Karnovsky. J. Cell Biol. 39:168A, 1968.

2. D.H. Kay. Techniques for Electron Microscopy, II ed., F.A. Davis Co., Philadelphia, pp. 260-261, 1967.

3. E.N. Albert and E. Fleischer. J. Histochem. Cytochem. 18: 697, 1970.

4. L.G. Caro, R.P. van Tubergen and J.A. Kolb. J. Cell Biol. 15:173, 1962.

B. Biochemistry of Connective Tissue

Analytical studies. Segments of lung will be pooled, weighed and assayed for DNA by the method of Schmidt (1), for hexosamine by a micromodification of the method of Rondle and Morgan (2) and for hydroxyproline by the method of Woessner (3). All determinations will be expressed as micrograms per milligram of dry weight and will be repeated at least two times on each sample for verification.

Assays for incorporation of radioisotopes. The samples (200 mg wet weight) will be sliced 1 mm thick with a McIlwain tissue chopper and incubated at 37°C for two hours in tubes containing 50 microcuries of thymidine-^{3}H and 20 microcuries of glycine-^{14}C; or 50 microcuries of cytidine-^{3}H and 20 microcuries of $^{35}SO_4$. After incubation the samples will be assayed for radioactivity (4), adjusted for simultaneous counting of double-labeled samples (5). The values obtained will be recorded as counts per minute (CPM) per milligram of dry weight and as CPM per milligram of DNA.

It will then be possible to calculate data for lung content of DNA, hexosamine, hydroxyproline, and hexosamine/hydroxyproline ratios. From the radioisotope incorporation data it will be possible to get estimates of the rates of DNA, RNA, Protein, and polysaccharide synthesis.

References

1. Schneider, W.C. Methods in Enzymology, ed. by S.P. Colowick and N.O. Caplan. Vol. 3, New York; Academic Press, 1956. p. 680.
2. C.J.M. Rondle, and W.T.J. Morgan. Biochem. J. 61:586, 1955.
3. Woessner, J.F., Jr. Arch. Biochem. Biophys. 93:440, 1961.
4. H. Yeager, Jr. and P.S. Hicks. Proc. Soc. Exp. Biol. Med. 141:1, 1972.
5. J.B. Birks. The Theory and Practice of Scintillation Counting. New York: Pergamon Press, 1964.

C. Biochemistry of Lipids

Labeled palmitic acid and labeled leucine will be used as precursors in the biosynthesis of lung lipid (with special emphasis on surfactant) and protein. The studies will be carried out in vivo and in the isolated perfused lung preparation.

Palmitic acid will be prepared for intravenous administration or tissue perfusion by the method of Darrah and Hedley-Whyte (1). Lipids will be extracted according to Folch et al. (2) and Dole (3). Total lipid concentration will be determined according to Rapport and Alonzo (4) and Dole and Meinertz (5). Phospholipid concentration will be determined according to Bartlett (6). The different phospholipid fractions will be separated by column and thin layer chromatography according to Mason et al. (7), Young and Tierney (8), Mangold (9) and Balint et al. (10).

The neutral lipid fractions will be separated by chromatographic procedures according to Hamosh and Scow (11) and Creech (12), Radioactivity in the isolated lipid fractions will be determined according to Hamosh et al. (13) and Snyder (14).

References

1. H.K. Darrah and J. Hedley-Whyte. J. Appl. Physiol. 34:205, 1973.
2. J. Folch, M. Lees and G.H. Sloam-Stanley. J. Biol. Chem. 226:497, 1957.
3. V.P. Dole. J. Clin. Invest. 35:150, 1956.
4. M.M. Rapport and M. Alonzo. J. Biol. Chem. 217:193, 1959.
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6. G.R. Bartlett. J. Biol. Chem. 234:466, 1959.
7. R.J. Mason, G. Huber and M. Vaughan. J. Clin. Invest. 51: 68, 1972.
8. Young, S.L. and D.F. Tierney. Am. J. Physiol. 222:1539, 1972.
9. Mangold, H.K. In Thin-Layer Chromatography. A Laboratory Handbook, ed. E. Stahl. New York: Academic Press, 1965, pp 137-86.
10. J.A. Balint, S. Bondurant and E.C. Kyriakides. Arch. Intern. Med. 127:740, 1971.
11. Hamosh, M. and R.O. Scow. J. Clin. Invest. 52:88, 1973.
12. B.G. Creech. J. Am. Oil Chem. Soc. 38:540, 1961.
13. M. Hamosh, T.R. Clary, S.S. Chernick and R.O. Scow. Biochim. Biophys. Acta 210:473, 1970.
14. F. Snyder. Anal. Biochem. 9:183, 1964.

D. Physiology

Determination of the elastic properties of intact lung

1. Pressure-volume measurements. The lung will be inflated to a transpulmonic pressure of 25 cm H_2O and the lung volume at

this distending pressure called "vital capacity". The lung will be deflated with a syringe by decrement of about one tenth of the vital capacity and the pressure at each volume recorded. This procedure will be repeated three times and the resulting curves plotted as volume/pressure curves. Pressure will be measured by a Hewlett-Packard 267B transducer, the signal amplified by a Hewlett-Packard 8805A carrier amplifier, the signal recorded on a Hewlett-Packard recorder. In saline filled lungs the pressure will be measured by a Statham P23V transducer.

2. Isolated lung preparation. After cannulation of the trachea, pulmonary artery and left atrium, the lung is suspended in a chamber thermostated at 37°C and saturated with water vapor. The perfusate has the basic composition of 4% albumin in Krebs-Ringer solution contained in a 37°C bath. The radioactive substrates are delivered into the line leading to the pulmonary artery with a metering pump (Sage Co.) The fluid from the left atrium is collected by gravity into iced test tubes in a modified fraction collector. The tracheal cannula is connected to a rodent respirator (Harvard Instrument Co.) with controlled volume and frequency. The chamber serves also as a volume plethysmograph and lung volume and transpulmonic pressure can be continuously monitored. Perfusate flow is monitored by a drop-counter on the collection site. Perfusion pressure is determined by the height of the perfusion unit, but pulsatile flow can be also generated by a peristaltic pump. The lung is suspended from the force-displacement transducer, so that lung weight can be also monitored continuously.

3. Determination of tensile strength. A longitudinal strip of lung tissue, trimmed and oriented, will be constrained on both ends and connected to a force-tension transducer and a linear displacement transducer. Stress and Strain will be measured and yield stress determined (Martin et al.*) This measurement is a good index of tissue elasticity.

4. Determination of area dependent surface activity. The surface active fraction from the lavage fluid will be separated by the method of Dickie et al. **. The fraction will be spread over saline and and the activity determined as a function of area using a modified Wilhelmy balance.

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*T. Sugihara, C.J. Martin and J. Hildebrandt. J. Appl. Physiol. 30:874, 1971

** K.J. Dickie, G.D. Massaro, V. Marshall and D. Massaro. J. Appl. Physiol. 34:606, 1973.

E. Morphometry and Stereology

For rapid evaluation of structural changes a computer technique known as pattern recognition based on the principle of evaluation of rapid electronic scanning (see Appendix II) will be used. In addition we are now in the process of developing a method of stereology for quick determination of the volume of inclusion bodies, such as lamellar bodies. The lamellar body is the storage unit for surfactant and determination of their size and quantity can be a useful index of intermediary metabolism of surfactant (Massaro*).

* G.D. Massaro and D. Massaro. J. Clin. Invest. 52:566, 1973.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

See appended.

11. Additional facilities required:

12. Biographical sketches of investigator(s) and other professional personnel (append):
appended.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).
Appended.

10. FACILITIES AVAILABLE

A. In the Department of Anatomy (Dr. Vidic)

1. A multidisciplinary laboratory fully equipped for micro-dissection and light microscopy
2. A fully equipped laboratory for processing of tissues and a fully equipped laboratory for cutting of tissues for electron microscopy and autoradiography
3. A fully equipped laboratory for initiation and maintenance of tissues in vitro
4. A fully equipped darkroom for electron and light micro-photography
5. Diamond knife
6. LKB - automatic ultra-microtome
7. AEI-EM 8 electron microscope

B. In the Department of Physiology and Biophysics (Dr. Hamosh)

1. A biochemistry laboratory, about 200 sq. ft. with specialized bench furniture, refrigerator, centrifuge, pH meter and perfusion apparatus.
2. A laboratory for lung mechanics of 200 sq. ft. with all the necessary transducers, amplifiers, recorders etc.
3. A laboratory for animal work, approx. 400 sq. ft with capabilities of housing, exposing, operating, etc.
4. Office space, about 200 sq. ft. to house all data processing equipment
5. Access to and regular use of liquid scintillation counters, preparatory centrifuges, spectrophotometers, cold room, etc. all in the department of physiology
6. In the National Biochemical Research Foundation, affiliated with the department and housed in the same building, access to IBM 360/44 computer with all accessories, primarily intended for pattern recognition work but also available for statistical use.

C. In the Department of Medicine (Dr. Yeager)

The laboratory area has about 320 sq. ft of laboratory space with appropriate sinks and utilities and 200 sq. ft of office space. There is access to liquid scintillation counters in the Department of Nuclear Medicine and Pharmacology and to a preparatiave ultra-centrifuge in the Pharmacology Department.

Equipment in the laboratory includes:

1. Spectrophotometer
2. pH meter
3. Vortex Genie Mixer
4. Homogenizer
5. Analytical Balance

6. Refrigerated centrifuge etc.

D. General Facilities

1. The "Vivarium". Accredited animal facility with resident veterinarian and animal care personnel with easy capability to house and care for animals projected in this study.

2. A central facility for storing and dispensing radioactive materials.

3. In addition to the above the university is equipped with a modern library, medical illustration service and data processing unit to handle all bibliographic and publication requirements.

BIOGRAPHICAL SKETCH

Mailing Address: Branislav Vidic, S.D., Department of Anatomy
Georgetown University School of Medicine,
3900 Reservoir Rd., NW, Washington, D.C. 20007

Date and Place of Birth:
Citizenship:
Marital Status:
Languages Spoken: English, French, German, Russian, Serbo-Croatian
Undergraduate Education: Sremska Mitrovica, Yugoslavia
Graduate Education: Faculty of Stomatology, University of Belgrade, Yugoslavia. Doctor of Stomatology 1959

Positions Held: Assistant in Anatomy, School of Medicine, University of Novi Sad, Yugoslavia, 1960-1962.
Visiting Assistant in Anatomy, School of Medicine, University of Basel, Switzerland, Summer 1961.
First Assistant in Anatomy, School of Medicine, University of Lausanne, Switzerland, 1962-1965.
Assistant and Associate Professor of Anatomy, School of Medicine, St. Louis University, St. Louis, Missouri, 1965-1971.
Associate Professor of Anatomy, School of Medicine, Georgetown University, Washington, D.C., 1971--

Member of :

Five Recent Publications:

1. Vidic, B., M.W. Rana and B.D. Bhagat. A reversible damage of the rat upper respiratory tract caused by cigarette smoking. Archs. Otolaryng. (AMA), in press.
2. Vidic, B. Structure and cytochemistry of the acinar cell in the rat maxillary gland. Am. J. Anat., 137:103-117, 1973.
3. Vidic, B., J.J. Taylor, M.W. Rana and B.D. Bhagat. The respiratory glandular system in the rat lateral nasal wall in normal and polluted environments. Anat. Anz., 130:83-85, 1972.
4. Vidic, B. and J.J. Taylor. The structure of the acinar cell and its relationship to the nerve terminals in the lateral nasal gland of the rat. Arch. Histol. Jap., 34:449-461, 1972.
5. Vidic, B.
The histochemical and microscopical differentiation of the respiratory glands around the maxillary sinus of the rat. Am. J. Anat., 132:491-514, 1971.

Just the address, not the name

BIOGRAPHICAL SKETCH

Paul Hamosh, M.D.
REDACTED

REDACTED

Graduate and Post-Graduate Education

1951-1957	Attended Medical School, Hebrew University, Hadassa Medical School, Jerusalem, Israel
1957-1963	Rotating internship and residency in Pathology, Internal Medicine and Chest Diseases
1963-1965	Fellow and Staff, Department of Medicine, B., "Ichilow" Municipal Hospital, Tel-Aviv, Israel
1966-1968	NIH Trainee in Cardio-Pulmonary Physiology, Department of Medicine, Georgetown University Medical School and Veterans Administration Hospital, Washington, D.C.

Professional Appointments

1968-1970	Lecturer in Physiology, Georgetown University Medical School
1970-1972	Assistant Professor of Medicine, George Washington University Medical School
1968-1970	Research Associate, Veterans Administration Hospital, Washington, D.C.
1970-1972	Director, Pulmonary Physiology Laboratory, Veterans Administration Hospital, Washington, D.C.
1972-date	Assistant Professor of Physiology and Biophysics, Georgetown University Medical School
1973-date	Assistant Professor of Medicine, Georgetown University Medical School
1973-date	Senior Cancer Research Internist, Veterans Administration Hospital/National Cancer Institute, Washington, D.C. (Part-time)

Honors

1972	Clinical Investigator, Veterans Administration (declined)

Societies

REDACTED

Five Recent Publications

1. Hamosh, P., Da Silva, A.M.T. Postural hypoxemia and erythrocytosis in two non-obese patients without manifest lung disease. Am. J. Med. 55:80, 1973.
2. Da Silva, A.M.T., and Hamosh, P. The effect of smoking a single cigarette on the small airways. J. Appl. Physiol. 34:361, 1973.
3. Gacad, G., and Hamosh, P. The lung in ankylosing spondylitis. Am. Rev. Resp. Dis. 107:286, 1973.

4. Hamosh, P. and Luchsinger, P.C. Respiratory mechanics and gas exchange in the squatting position. Am. Rev. Resp. Dis. 102:112, 1970.

5. Hamosh, P., Luchsinger, P.C. Maximum expiratory flow in isolated liquid-filled lungs. J. Appl. Physiol. 25:485, 1968.

BIOGRAPHICAL SKETCH — Henry Yeager, Jr., M.D.

Personal Information

Place of Birth:
Date of Birth: REDACTED
Marital Status:

Address: Georgetown Univ . Hospital Home: REDACTED
3800 Reservoir Road, NW
Washington, D.C. 20007
(202) 625-7027

Education

Southern Methodist University (Phi Beta Kappa) REDACTED
Johns Hopkins University

Postgraduate Education

Internship, Vanderbilt Hospital, Nashville, Tenn.	1957-1958
Residency in Internal Medicine, Parkland Memorial Hospital, Dallas, Texas	1958-1960
Fellowship in Arthritis, Dr. Morris Ziff, Southwestern Medical School, Dallas, Texas	1960-1961
Fellowship in Pulmonary Disease, Dr. Charles A. LeMaistre, Southwestern Medical School, Dallas, Texas	1963-1964
Fellowship in Pulmonary Disease, Dr. H.O. Sieker, Duke University School of Medicine, Durham, North Carolina	1967-1968

Specialty Board: Diplomate, American Board of Internal Medicine 1965

Professional Experience

Instructor, Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas	1964-1965
Clinical Instructor, Internal Medicine, University of Texas, Southwestern Medical School, Dallas, Tex.	1965-1967
Consultant Internist in Project HOPE, Nicaragua, Central America (Sept.-Nov.)	1966
Research Associate, V.A. Hospital, Washington, D.C.	1968-1970
Assistant Professor of Medicine, George Washington University School of Medicine, Washington, D.C.	1968-1970
Staff Physician, Medical Service, V.A. Hospital, Houston, Texas	1970-1972
Assistant Professor of Medicine, Baylor College of Medicine, Houston, Texas	1970-1972
Assistant Professor of Medicine, Georgetown University School of Medicine, Washington, D.C.	1972--

Medical License: Maryland, Texas and District of Columbia

Professional Societies

REDACTED

1960
1960
1963
1967
1971

Military Service

U.S. Army, Captain in Medical Corps, Ft. Hood, Texas 1961-1963

Five Recent Publications

1. Yeager, H., Jr. and Massaro, D. Glucose metabolism and glycoprotein synthesis in lung slices. J. Appl. Physiol. 32:477, 1972.

2. Yeager, H., Jr. Tracheobronchial secretions. Am. J. Med. 50:493, 1971.

3. Yeager, H., Jr., Massaro, D., and Massaro, G. Glycoprotein synthesis by the trachea. Am. Rev. Resp. Dis. 103:188, 1971.

4. Massaro, D., Massaro, G., Keleher, K., and Yeager, H., Jr. Alveolar cells: depression of protein synthesis during phagocytosis. Am. J. Physiol. 218:1533, 1970.

5. Yeager, H., Jr. Alveolar cells: depressant effect of cigarette smoke on protein synthesis. Proc. Soc. Exper. Biol. Med. 131:247, 1969.

BIOGRAPHICAL SKETCH

Margit Hamosh, Ph.D.

REDACTED

Graduate and Post-Graduate Education

REDACTED

Attended Hebrew University
M.Sc. in Microbiology
Doctoral Fellow, Department of Biochemistry, Hebrew University Medical School
Ph.D. in Biochemistry
Postdoctoral work in Department of Biochemistry, Hebrew University

Appointments

1964	Lecturer in Biochemistry, Hebrew University Medical School
1965-date	Visiting Scientist at National Institute of Arthritis, Metabolism and Digestive Diseases

Five Recent Publications

1. M. Hamosh and R.O. Scow. Lingual lipase and its role in the digestion of dietary lipid. J. Clin. Invest. 52:88, 1973.

2. M. Hamosh and R.O. Scow. Lipoprotein lipase activity in guinea pig and rat milk. Biochim. Biophys. Acta 231:283, 1971.

3. Margit Hamosh and Robert O. Scow: Plasma triglyceride and lipoprotein lipase activity in pregnant and lactating rats. Nutrition, Proc. VIII International Congress on Nutrition, Prague 1969, Ed. J. Masek et al., Excerpta Medica, ICS No. 213, 207-209, 1970.

4. M. Hamosh, T.R. Clary, S.S. Chernick and R.O. Scow. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim. Biophys. Acta 210:473, 1970.

5. M. Hamosh, M. Lesch, J. Baron and S. Kaufman: Enhanced protein synthesis in a cell-free system from hypertrophied skeletal muscle. Science 157:935, 1967.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Dr. Branislav Vidic	25	--
Dr. Paul Hamosh	15	--
Dr. Henry Yeager, Jr.	Consultant	--
Dr. Margit Hamosh	Consultant	--
Research Assistant (to be hired)* (M.S. or Ph.D.)	100	R
Technical Technician*	100	R
Administrative Assistant (data processing)*	25	

* 11% Fringe Benefits included.

Sub-Total for A	R

B. Consumable supplies (by major categories)

1. Experimental animals (purchase and maint.)	2,500.
2. Chemicals for ultrastructure	750.
3. Radioactive isotopes	750.
4. Chemicals for biochemistry	1,250.
5. Photographic supplies	1,500.
6. Glassware, miscellaneous	500.
Sub Total for B	7,250.

C. Other expenses (itemize)

Consultant, maintenance of equipment	1,000.
Data processing, computer time	500.
Travel	1,000.
Publication	500.
Sub-Total for C	3,000.
Running Total of A + B + C	33,250.

D Permanent equipment (itemize)

Estimated cost of exposure facility	2,500.
Sub-Total for D	2,500.
E	4,988.
Total request	$40,738.

E Indirect costs (15% of A+B+C)

15. Estimated future requirements.

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	7,975.	3,300.	--	5,486.	42,061.
Year 3		8,773.	3,630.	--	6,034.	46,267

16. Other sources of financial support.
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
A reparable damage of the maxillary epithelium and gland of the rat caused by cigarette smoking.	PHS grant RR5360-11	1,000.	9/30/71-8/31/73
The effect of whole cigarette smoke on the oral tissue.	GRS grant. Georgetown Univ. Dental School	800.	3/14/72-8/31/73
Ultrastructure and metabolism of isolated perfused lung.	Washington Heart Assoc. grant 3-282-777	7,532.	7/1/73-6/30/74
Surfactant metabolism as a function of ventilation	Washington Heart Assoc. grant 3-287-785	7,528.	1/1/73-12/31/73
Effect of mechanical stress on the elastic properties of the lung in papain induced emphysema	GRS-NIH 3302-113 Georgetown Univ. Med. School	2,500.	1/1/73-12/31/73
Quantitation of lung cancer on chest films by computer	Contract with V.A.	10,800.	1/1/73-12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Computerized chest X-ray followup in cancer therapy	National Cancer Inst.	80,460.	1/1/74-12/31/76
Pediatric lung development program project	National Heart and Lung Institute	Approx. 200,000.	7/1/74-6/30/79

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Branislav Vidic, S.D.

Signature [signature] Date 7/31/1973

Telephone — R — Area Code Number Extension

Checks payable to

Georgetown University BJB

Mailing address for checks Sam A. Kimble
Georgetown University
37th and O Streets, N.W.
Washington, D.C. 20007

Responsible officer of institution

Typed Name Guerry R. Smith

Title Acting Administrator, Sponsored Programs

Signature [signature] Date 7/31/73

Telephone — R — Area Code Number Extension

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